Disturbed vitamin A metabolism in chronic liver disease and relevance for therapy

Saeed, Ali

Chapter 1

Introduction and aim of the thesis

Ali Saeed[1,2], Klaas Nico Faber[1,3]

[1]Department of Gastroenterology and Hepatology, [3]Laboratory Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

[2]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan

1.1. INTRODUCTION

Vitamin A is an essential nutrient, principally meaning that humans and animals fully depend on dietary sources to supply key physiological processes in the body with this vitamin. Plants synthesize carotenoids, like alpha- and beta-carotene, that give the characteristic color to carrot and corn and are the primary source of vitamin A. Carotenoids absorb light energy for photosynthesis, but also protect chlorophyll from light-inflicted photo damage. The typical color change of leaves in fall is a result of degradation of chlorophyll (green pigment) into colorless tetrapyrroles, at the same time revealing the color of other pigments of which carotenoids (from yellow to orange) are often abundantly present [1,2]. Animals eat the plants and roots containing the carotenoids and most of it this “pro-vitamin A” is absorbed in the small intestine. As carotenoids are lipophilic compounds, efficient intestinal absorption depends on the presence of sufficient bile acids, which are produced in the liver and are released in the small intestine for that purpose: keeping fat-soluble compounds “in solution”, either for uptake or effective excretion [3]. Most of the carotenoids are converted to retinol in the intestine, which is subsequently esterified to long chain fatty acids (LCFAs), such as palmitate and stearate, and released to the bloodstream for distribution to peripheral tissues that need vitamin A [3,4]. As quite a bit of vitamin A accumulates in fat tissue, many meat- and/or organ-based foods are also an important nutritional source of vitamin A for humans [5,6]. Most of the “dietary” vitamin A is, however, routed to the liver where excess vitamin A is stored and typically contains over 80% of the total vitamin A pool in a healthy individual [5]. The liver is “in control” of supplying the body with sufficient vitamin A in times when dietary intake is low, and it has an impressive capacity to maintain normal vitamin A homeostasis. Even in the absence of any vitamin A intake, it may take months to years before humans experience vitamin A deficiency, also called hypovitaminosis A. In the meantime, stable blood levels of retinol are maintained at around 2 µmol/L independent of the vitamin A pool size in the liver [7]. The typical symptom of vitamin A deficiency is night blindness, which is a result of the impaired production of rhodopsin that depends on specific vitamin A metabolites [8]. Still, vitamin A deficiency leads to many more problems as it also impairs skin and tissue regeneration, immune control, metabolic control, fertility as well as predisposes for cancer [9]. Retinol is secreted from the liver to the blood circulation bound to retinol binding protein 4 (RBP4). Retinol is taken up by tissues requiring vitamin A and

converted to retinoic acids, which are the active metabolites of vitamin A [10]. Most of the processes that require vitamin A are actually controlled by retinoic acids that activate ligand-dependent transcription factors, e.g. retinoic acid-activated receptors (RARs) and retinoid X receptors (RXRs). Retinol is the mother compound that is converted to different forms of retinoic acids, which in the end largely determines the ultimate effect in physiological processes as all-trans retinoic acid (atRA) is a high-affinity ligand for RARs, while 9-cis retinoic acid (9cRA) and 9-cis-13,14 dihydroretinoic acid (9cDHRA) activate RXRs [10,11]. Hepatic vitamin A uptake, storage and release depends on a complex interplay between different cell types in the liver, in particular the hepatocytes and hepatic stellate cells [5]. Up to 80-85% of the total liver cell mass is taken by hepatocytes and they are considered to determine most of the liver functions, e.g. controlling glucose and lipid metabolism, production of blood proteins and detoxification [11,12]. Vitamin A absorbed in the intestine, mostly retinyl esters in chylomicron remnants, first arrives in hepatocytes where it is converted to retinol and secreted to the blood bound to RBP4. Excess retinol is taken up by HSC and converted to retinyl esters again for long term storage [10]. Many details of how hepatocytes and HSC communicate to maintain the stable retinol levels in blood are, however, still unclear. Chronic liver diseases, including viral, metabolic, immune-mediated and obstructive forms, are often associated with impaired bile acid metabolism and/or bile flow, which as a result affects intestinal absorption of fat-soluble nutrients, including vitamin A [13]. Moreover, the progression of liver diseases also leads to fibrosis, which may progress to cirrhosis and liver cancer. HSC are considered to be the main liver cell type causing fibrosis. The vitamin A-containing “quiescent” HSC that are characteristic for the healthy liver undergo a dramatic phenotypic and functional transdifferentiation in the chronically-injured liver [12]. They transform to highly proliferative, migratory and extracellular matrix-producing “activated’ HSC that lose their vitamin A content in this process [14]. Both pathological processes contribute to a reduction in the hepatic vitamin A pool, that may even progress to systemic vitamin A deficiency (VAD), which is defined as serum retinol levels below 0.7 μmol/L [15]. The prevalence of VAD in chronic liver diseases varies between studies, which may lie in differences in disease etiology, group size, patient age and also the used definition of “vitamin A deficiency”. Some studies the strict cut-off of serum retinol of 0.7 μM, while others also include the range of 0.7-1.05 μM as “vitamin A inadequate”. Still, vitamin A deficiency has been

reported for 20-40% of patients with primary biliary cholangitis (PBC) or primary sclerosis cholangitis (PSC) [16–19], while up to 70% of pediatric patients with biliary atresia may develop VAD [20]. Moreover, non-alcoholic fatty liver disease (NAFLD) and related pathologies like obesity, type 2 diabetes and metabolic syndrome, have repeatedly been shown to be associated with impaired systemic vitamin A status, including lowered serum retinol levels and/or elevated serum RBP4 levels [21]. Still, it remains largely unknown whether 1) this is due to loss of vitamin A from the liver or a change in hepatic vitamin A metabolism and 2) whether impaired vitamin A status actually contributes to disease progression. Recent observations indicate that impaired vitamin A metabolism may contribute to chronic liver disease, including NAFLD. One is that genome-wide association studies have now identified 2 genes associated with NAFLD that encode enzymes involved in vitamin A metabolism, e.g. the retinyl ester hydrolase patatin-like phospholipase domain-containing protein 3 (PNPLA3) and the retinol dehydrogenase hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) [22–24]. Secondly, vitamin A metabolites, especially atRA and retinyl aldehyde as well as synthetic ligands for RARs, have therapeutic effects in animal models of NAFLD [21].

Thus, there are ample indications that chronic liver diseases are linked to impaired vitamin A status, but we know very little mechanistic details. Such knowledge is required to evaluate the therapeutic potential of vitamin A and/or specific vitamin A metabolites in these diseases.

1.2. THE AIM OF THE THESIS

The overall aim of this thesis is the delineate molecular mechanism that are involved in hepatic vitamin A metabolism in chronic liver diseases and how this may be affected by drugs currently studied for the treatment of NAFLD. In **Chapter 2**, we first provide a comprehensive overview of the interrelationship between bile acid and vitamin A homeostasis and how this is related to various chronic liver diseases**.** In **Chapter 3**, we analyzed the role of the hormone sensitive lipase (HSL) as a retinyl ester hydrolase and HSC and how this role may change during HSC transdifferentiation. In **Chapter 4**, we summarize the current knowledge about vitamin A metabolism in NAFLD and its putative role in disease progression, as well as the therapeutic potential of vitamin A metabolites. From this critical review, we conclude that there is actually quite a bit of controversy about what is going on with vitamin A metabolism in the fatty liver. Thus, in **Chapter 5** we analyzed vitamin A

metabolism in 2 animal models of NAFLD, e.g. the high fat-high cholesterol diet model, as well as the *Leptin*^{ob} mutant (*ob/ob*) genetic model. We quantified retinol and retinol ester levels in the liver and found that technical aspects of the analytical procedures for these compounds heavily affect the experimental outcome. Using complementary approaches we obtained consistent results that reveal that NAFLD does not lead to true vitamin A deficiency, but to cell type-specific rearrangements in vitamin A metabolism. In **Chapter 6,** we analysed hepatic vitamin A metabolism in glycogen storage disease type 1a (GSD Ia), a syndrome caused by mutations in the catalytic subunit of glucose-6-phosphatase *(G6PC)*. Increased liver fat is a typical symptom of GSD Ia and we wanted to know whether this similarly affects vitamin A metabolism as in NAFLD. Serum retinol levels were determined of GSD Ia patients, as well as hepatic vitamin A metabolism in liver-specific *G6pc* knock-out mice. Indeed, vitamin A metabolism is impaired in the absence of G6PC, but in a clear different manner than observed for the NAFLD models in chapter 5. In **Chapter 7**, we analyzed whether the farnesoid X receptor (FXR), which is the bile acid sensor and therapeutic target in NAFLD, affects hepatic vitamin A metabolism. We quantified retinol and retinyl esters in whole body- and intestine-specific FXR-null mice, as well as after reintroduction of hepatic FXR in whole body FXR-null mice. Moreover, wild type animals were treated with obeticholic acid (OCA), a high-affinity ligand of FXR and currently under investigation for the treatment of NAFLD, and the normal bile acid cholic acid (CA) to determine their effect on hepatic vitamin A metabolism. Again, hepatic vitamin A metabolism was strongly affected by the absence of FXR as well as the ligand-activation of FXR. Finally, in **Chapter 8**, we summarize the results obtained in the experimental studies in this thesis and present and provide an outlook for future directions to target vitamin A metabolism in the treatment of chronic liver diseases.

REFERENCES

[1] M. Shumskaya, E.T. Wurtzel, The carotenoid biosynthetic pathway: thinking in all dimensions, Plant Sci. Int. J. Exp. Plant Biol. 208 (2013) 58–63. doi:10.1016/j.plantsci.2013.03.012.

[2] J.-H. Park, S. Jung, Perturbations of carotenoid and tetrapyrrole biosynthetic pathways result in differential alterations in chloroplast function and plastid signaling, Biochem. Biophys. Res. Commun. 482 (2017) 672–677. doi:10.1016/j.bbrc.2016.11.092.

[3] E. Reboul, Absorption of vitamin A and carotenoids by the enterocyte: focus on transport proteins, Nutrients. 5 (2013) 3563–3581. doi:10.3390/nu5093563.

[4] T. Bohn, C. Desmarchelier, S.N. El, J. Keijer, E. van Schothorst, R. Rühl, P. Borel, β-Carotene in the human body: metabolic bioactivation pathways - from digestion to tissue distribution and excretion, Proc. Nutr. Soc. 78 (2019) 68–87. doi:10.1017/S0029665118002641.

[5] S.M. O'Byrne, W.S. Blaner, Retinol and retinyl esters: biochemistry and physiology, J. Lipid Res. 54 (2013) 1731–1743. doi:10.1194/jlr.R037648.

[6] R. Schreiber, U. Taschler, K. Preiss-Landl, N. Wongsiriroj, R. Zimmermann, A. Lass, Retinyl ester hydrolases and their roles in vitamin A homeostasis, Biochim. Biophys. Acta. 1821 (2012) 113–123. doi:10.1016/j.bbalip.2011.05.001.

[7] R. Blomhoff, M.H. Green, T. Berg, K.R. Norum, Transport and storage of vitamin A, Science. 250 (1990) 399–404.

[8] C.M. Kemp, S.G. Jacobson, D.J. Faulkner, R.W. Walt, Visual function and rhodopsin levels in humans with vitamin A deficiency, Exp. Eye Res. 46 (1988) 185–197.

[9] E.M. Wiseman, S. Bar-El Dadon, R. Reifen, The vicious cycle of vitamin a deficiency: A review, Crit. Rev. Food Sci. Nutr. 57 (2017) 3703–3714. doi:10.1080/10408398.2016.1160362.

[10] W.S. Blaner, Y. Li, P.-J. Brun, J.J. Yuen, S.-A. Lee, R.D. Clugston, Vitamin A Absorption, Storage and Mobilization, Subcell. Biochem. 81 (2016) 95–125. doi:10.1007/978-94-024-0945-1_4.

[11] A. Saeed, M. Hoekstra, M.O. Hoeke, J. Heegsma, K.N. Faber, The interrelationship between bile acid and vitamin A homeostasis, Biochim. Biophys. Acta. 1862 (2017) 496–512. doi:10.1016/j.bbalip.2017.01.007.

[12] Z. Kmieć, Cooperation of liver cells in health and disease, Adv. Anat. Embryol. Cell Biol. 161 (2001) III–XIII, 1–151.

[13] C. Freund, D.N. Gotthardt, Vitamin A deficiency in chronic cholestatic liver disease -is vitamin A therapy beneficial ?, Liver Int. Off. J. Int. Assoc. Study Liver. (2017). doi:10.1111/liv.13433.

[14] J.X. Jiang, N.J. Török, Liver Injury and the Activation of the Hepatic Myofibroblasts, Curr. Pathobiol. Rep. 1 (2013) 215–223. doi:10.1007/s40139-013-0019-6.

[15] World Health Organization, Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes, Eileen Brown, James Akré, World Health Organization: Geneva, Switzerland, 1996. http://www.who.int/nutrition/publications/micronutrients/vitamin_a_deficiency/WHO_NUT_96.10/en/.

[16] J.R. Phillips, P. Angulo, T. Petterson, K.D. Lindor, Fat-soluble vitamin levels in patients with primary biliary cirrhosis, Am. J. Gastroenterol. 96 (2001) 2745–2750. doi:10.1111/j.1572-0241.2001.04134.x.

[17] S.J. Muñoz, J.E. Heubi, W.F. Balistreri, W.C. Maddrey, Vitamin E deficiency in primary biliary cirrhosis: gastrointestinal malabsorption, frequency and relationship to other lipid-soluble vitamins, Hepatol. Baltim. Md. 9 (1989) 525–531.

[18] B.L. Shneider, J.C. Magee, J.A. Bezerra, B. Haber, S.J. Karpen, T. Raghunathan, P. Rosenthal, K. Schwarz, F.J. Suchy, N. Kerkar, Y. Turmelle, P.F. Whitington, P.R. Robuck, R.J. Sokol, Childhood Liver Disease Research Education Network (ChiLDREN), Efficacy of fat-soluble vitamin supplementation in infants with biliary atresia, Pediatrics. 130 (2012) e607-614. doi:10.1542/peds.2011-1423.

[19] R.A. Jorgensen, K.D. Lindor, J.S. Sartin, N.F. LaRusso, R.H. Wiesner, Serum lipid and fat-soluble vitamin levels in primary sclerosing cholangitis, J. Clin. Gastroenterol. 20 (1995) 215–219.

[20] Y.-M. Shen, J.-F. Wu, H.-Y. Hsu, Y.-H. Ni, M.-H. Chang, Y.-W. Liu, H.-S. Lai, W.-M. Hsu, H.-L. Weng, H.-L. Chen, Oral absorbable fat-soluble vitamin formulation in pediatric patients with cholestasis, J. Pediatr. Gastroenterol. Nutr. 55 (2012) 587–591. doi:10.1097/MPG.0b013e31825c9732.

[21] A. Saeed, R.P.F. Dullaart, T.C.M.A. Schreuder, H. Blokzijl, K.N. Faber, Disturbed Vitamin A Metabolism in Non-Alcoholic Fatty Liver Disease (NAFLD), Nutrients. 10 (2017). doi:10.3390/nu10010029.

[22] Y. Ma, O.V. Belyaeva, P.M. Brown, K. Fujita, K. Valles, S. Karki, Y.S. de Boer, C. Koh, Y. Chen, X. Du, S.K. Handelman, V. Chen, E.K. Speliotes, C. Nestlerode, E. Thomas, D.E. Kleiner, J.M. Zmuda, A.J. Sanyal, NASH CRN, N.Y. Kedishvili, T.J. Liang, Y. Rotman, HSD17B13 is a Hepatic Retinol Dehydrogenase Associated with Histological Features of Non-Alcoholic Fatty Liver Disease, Hepatol. Baltim. Md. (2018). doi:10.1002/hep.30350.

[23] J.A. Del Campo, R. Gallego-Durán, P. Gallego, L. Grande, Genetic and Epigenetic Regulation in Nonalcoholic Fatty Liver Disease (NAFLD), Int. J. Mol. Sci. 19 (2018). doi:10.3390/ijms19030911.

[24] A.A. Ashla, Y. Hoshikawa, H. Tsuchiya, K. Hashiguchi, M. Enjoji, M. Nakamuta, A. Taketomi, Y. Maehara, K. Shomori, A. Kurimasa, I. Hisatome, H. Ito, G. Shiota, Genetic analysis of expression profile involved in retinoid metabolism in non-alcoholic fatty liver disease, Hepatol. Res. Off. J. Jpn. Soc. Hepatol. 40 (2010) 594–604. doi:10.1111/j.1872-034X.2010.00646.x.